

Application No.: 10/091,567

Docket No.: NEL-006

AMENDMENTS TO THE CLAIMS

Please cancel claims 13 and 16 without prejudice or disclaimer to its underlying subject matter. Please amend claims 10-12, 14-15 and 17 as set forth below:

1-7. (canceled)

8. (previously presented) A deoxyribonucleic acid (DNA) vaccine comprising a liposome-encapsulated plasmid containing a gene encoding for hemagglutinin protein.

9. (new) The vaccine as claimed in Claim 8 deliverable to a respiratory tract using intranasal administration and/or by aerosol inhalation.

10. (currently amended) The deoxyribonucleic acid (DNA) vaccine of claim 8. Use of the vaccine of claim 8 for preventing and/or treating wherein the vaccine prevents or treats an influenza virus infection.

11. (currently amended) The deoxyribonucleic acid (DNA) vaccine of claim 8. Use of the vaccine of claim 8 for eliciting wherein the vaccine elicits long-lasting protective antiviral immune responses against influenza viruses.

12. (currently amended) A plasmid vector construct pCI-HA10 comprising a gene encoding for hemagglutinin protein and capable of expressing said hemagglutinin protein in a host, said plasmid vector construct encapsulated in a liposome formulation.

13. (canceled)

14. (currently amended) A method for constructing a plasmid pCI-HA10 comprising the following steps:

- (1) amplifying hemagglutinin gene from viral and mRNA with PCR;
- (2) inserting and ligating the hemagglutinin gene into a pCI vector;
- (3) transforming the resulting vector into competent E.coli DH5α cells;

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(4) transcribing and translating of pCI-HA10; and

(5) preparing and purifying pCI-HA10 by bulk preparation method~~The method of constructing the plasmid pCI-HA10 of claim 13, and further comprising the step of encapsulating the plasmid pCI-HA10 in a liposome formulation, said liposome formulation comprising of 7% 1,2 dioleoyl-3-dimethylammonium chloride (DODAC), 78% 1,2-dioleoyl-sn-glycerol-3-phospho-ethanolamine (DOPE) and 15% polyethylene glycol C8 ceramide (PEG<sub>2000</sub>C<sub>8</sub>CER).~~

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15. (currently amended) A method for constructing a plasmid pCI-HA10 comprising the following steps:

(1) amplifying hemagglutinin gene from viral and mRNA with PCR;

(2) inserting and ligating the hemagglutinin gene into a pCI vector;

(3) transforming the resulting vector into competent E.coli DH5 $\alpha$  cells;

(4) transcribing and translating of pCI-HA10; and

(5) preparing and purifying pCI-HA10 by bulk preparation method~~The method of constructing the plasmid pCI-HA10 of claim 13, and further comprising the step of encapsulating the plasmid pCI-HA10 in a liposome formulation, comprising the following steps:~~

(1) preparing 7% DODAC, 78% DOPE, and 15% PEG<sub>2000</sub>C<sub>8</sub>CER at 10mg/ml concentrations to form a lipid film at 50 °C for 2h under vacuum;

(2) incubating the lipid film at 50 °C for 2h under vacuum;

(3) reconstituting the lipid film with distilled water and 1M  $\beta$ -octylglucanopyranoside detergent at 20% of the total preparation volume;

(4) adding the plasmid DNA to the lipid film at a concentration of 400  $\mu$ g DNA/ml of 10 mg/ml;

(5) transferring the reconstituted preparation into dialysis tubing and dialyzing in 1X HEPES buffer solution (150 mM NaCl, 20 mM Hepes, pH 7.4) at 23 °C for 15 h; and

(6) removing the free, non-encapsulate DNA from encapsulated DNA on a DEAE Sepharose CL-6B anion exchange column.

16. (cancelled)

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17. (currently amended) The method of constructing A method of delivering the liposome-encapsulated the plasmid pCI-HA10 of claim +6-15, further comprising the step of delivering the liposome-encapsulated pCI-HA10 to a respiratory tract using intranasal administration, and/or aerosol inhalation to eliciting protective antiviral immune responses to influenza viruses.

18. (previously presented) A method for preventing and/or treating influenza virus infection, comprising administering to a patient in need thereof a pharmaceutically effective amount of the vaccine of claim 8.

19. (previously presented) A method for eliciting long-lasting protective antiviral immune responses against influenza viruses, comprising administering to a patient in need thereof a pharmaceutically effective amount of the vaccine of claim 8.